REMARKS

Reconsideration is requested.

The Examiner interview of October 19, 2005 is acknowledged, with appreciation. The Interview Summary is accurate in its brief statement of the issues discussed.

The Examiner's identification of allowable subject matter on page 5 of the Office Action dated July 18, 2005, is acknowledged, with appreciation.

Claims 1-101 have been canceled, without prejudice. Claims 102-119 have been added and are pending. Support for the pending claims may be found throughout the specification. No new matter has been added.

The specification has been amended to include the sequence identifier as suggested by the Examiner on page 2 of the Office Action dated July 18, 2005.

The objection of claims 98 and 101 stated on page 2 of the Office Action of July 18, 2005, is moot in view of the above amendments. The claims have been drafted with the Examiner's comments in mind.

The Section 112, first paragraph "written description", rejection of claims 98-101 is moot in view of the above amendments. The Section 112, first paragraph "enablement", rejection of claims 98-101 is moot in view of the above amendments.

The pending claims are submitted to be supported by an enabling disclosure which adequately describes the claimed invention. Consideration of the following and the attached in this regard is requested.

Written Description

In rejecting claims 98, 99 and 101, for an alleged lack of written description support, the Examiner has asserted that:

> "the claims are directed to a crystal of P450 3A4 from human or any other mammal".

However, as discussed with the Examiners during the interview and as noted in the attached Declaration of Dr. Williams (Declaration, paragraph 7), P450 nomenclature gives each P450 a family (e.g. 3), a sub-family (e.g. 3A) and an individual gene (e.g. 3A4) based on sequence identity. Thus only identical genes and their alleles can be given the same name, and reference in the claims to 3A4 will be understood by a person of ordinary skill in the art to be a reference to a ***human*** P450 protein, and not to a P450 from another mammalian species.

The Examiner has further stated that SEQ ID NO:2 is a non-glycosylated protein expressed in *E. coli*. It appears that the Examiner is under the impression that if expressed in a eukaryotic host system, 3A4 would be a glycosylated protein. As discussed with the Examiners during the interview, 3A4 is not known to be a glycosylated protein in its natural environment. See also attached Declaration of Dr. Williams, paragraph 8.

As discussed with the Examiners during the interview, SEQ ID NO:2 represents the wild-type sequence with an internal deletion of residues 3-24 (as described on page 10 lines 31-32 of the present application) with a C-terminal four his tag. The Examiner has asserted on page 2 of the Office Action of July 18, 2005, that

> "residues 3-20 of the native human P450 3A4 is substituted by SEQ ID NO: 3"

In fact, SEQ ID NO:3 (16 amino acids) represents amino acids 1-2 and 25-38 of the wild type sequence. Therefore the replacement of residues 1-38 of the native human P450

3A4 with SEQ 3 results in the N-terminal sequence of SEQ ID: 2 which represents the wild-type sequence of 3A4 with deletion of residues 3-24.

The Examiner has further asserted that the specification:

> "only provides teaching for the crystallization of SEQ ID NO: 2 under two sets of crystallization conditions which produce crystals for structure determination". See page 2 of the Office Action of July 18, 2005.

It is respectfully pointed out that in addition to the extensive teaching of numerous ranges for crystallization of 3A4 crystals, there are in fact ***thirty four*** sets of specific conditions for crystallization which are described in the application. Consideration of the following in this regard is requested.

With regard to the general teaching, page 11, lines 15-22 sets out a method of making a 3A4 protein crystal comprising a 3A4 P450 core sequence as defined in the preceding part of the specification. As set out in the accompanying Declaration of Dr. Williams, an expert in the field of P450 protein crystallography (paragraph 18), this part of the description is addressed to a person of skill in the art who will understand this teaching as applying across the genus of the P450 3A4s claimed, including allelic variants of 3A4 having 1 or 2 amino acid substitutions (Declaration of Dr. Williams, paragraph 18).

Dr. Williams also notes in ¶18 of the attached Declaration that the specification sets out further detailed conditions starting at page 13 line 36 to page 15 line 35 is entitled "*(iii) Crystallization of 3A4*". The section starts:

> To produce crystals of 3A4 protein the final protein is, conveniently, concentrated to 10-60, e.g. 20-40 mg/ml in 10-100 mM potassium phosphate

> with high salt (e.g. 500 mM NaCl or KCl) by using concentration devices which are commercially available. Crystallisation of the protein is set up by the 0.5-2 µl hanging drop method and the protein is crystallised by vapour diffusion at 5-25 °C against a range of vapour diffusion buffer compositions.
>
> Typically the vapour diffusion buffer comprises 0 - 27.5%, preferably 2.5-27.5% PEG 1K-20 K, preferably 1-8K or PEG 2000MME-5000MME, preferably PEG 2000 MME, or 0-10% Jeffamine M-600 and/or 5-20%, e.g. 10-20% propanol or 15-20% ethanol or about 15%-30%, e.g. about 15% 2-methyl-2,4-pentanediol (MPD), optionally with 0.01 M -1.6 M salt or salts and/or 0-0.15, e.g. 0-0.1, M of a solution buffer and/or 0-35%, such as 0-15%, glycerol and/or 0-35% PEG300-400; but preferably:
>
> 10-25% PEG 1K-8K or PEG 2000MME or 0-10% Jeffamine M-600 and/or 5-15%, e.g. 10-15%, propanol or ethanol, optionally with 0.1 M -0.2 M salt or salts and/or 0-0.15, e.g. 0-0.1 M solution buffer and/or PEG400, but more preferably:
>
> 15-20% PEG 3350 or PEG 4000 or PEG 2000MME or 0-10% Jeffamine M-600 or 5-15%, e.g. 10-15% propanol or ethanol, optionally with 0.1 M -0.2 M salt or salts and/or 0-0.15 M solution buffer.

The text continues with detailed written description of suitable salts and other features of crystallization conditions which may be used. Thus, the specification sets out a clear written description of conditions under which 3A4 crystals may be produced.

With regard to specific conditions, page 57 starting at line 20 teaches that crystals of 3A4 were grown using the hanging drop vapor diffusion method:

> Protein at 40 mg/ml in 10mM Kpi pH 7.4, 0.5 M KCl, 2mM DTT, 1mM EDTA. 20% glycerol, was mixed in a 1:1 ratio, using 0.5ul drops, with a reservoir solution. The crystals of 3A4 grew over a reservoir solution containing 0.1 M HEPES pH

7.5, 0.2 M sodium chloride, 30% PEG 400. *[Page 57 lines 21-24]*

Having described this first condition, the specification continues from page 57 line 26 with the following twenty nine alternative conditions:

Alterative conditions are listed below:

0.1 M HEPES pH 7.5, 0.2 M sodium chloride, 30% PEG 400

0.05 M HEPES pH 7.5, 0.2 M sodium chloride, 35% PEG 400

0.05 M HEPES pH 7.5, 0.2 M sodium chloride, 30% PEG 400

0.15 M Imidazole-HCl pH 8, 10% 2-propanol

0.1 M 2-(N-cyclohexylamino)ethanesulfonic acid (CHES) pH 9.5, 30% PEG 400

0.15 M Hepes - Na pH 7.5, 5% IPA, 10% Peg 4000

0.1 M phosphate-citrate pH 4.2, 1.6 M NaH2PO4/ 0.4M K2HPO4

0.1 M citrate pH 5.5, 0.2 sodium chloride, 1.0 M Ammonium phosphate

0.2 M Lithium chloride, 20% PEG 3350

0.2 M Potassium chloride , 20% PEG 3350

0.2 M Sodium formate , 20% PEG 3350

0.2 M Potassium formate , 20% PEG 3350

0.2 M Ammonium formate , 20% PEG 3350

0.2 M Lithium acetate, 20% PEG 3350

0.2 M Potassium chloride, 20% PEG 3350

0.2 M Sodium formate , 20% PEG 3350

0.2 M Lithium acetate , 20% PEG 3350

0.2 M Sodium acetate , 20% PEG 3350

0.2 M Potassium acetate , 20% PEG 3350

0.2 M Ammonium acetate, 20% PEG 3350

0 .1 M HEPES pH 7.5, 0.2 M sodium chloride , 30% PEG 400

0.1 M HEPES pH 7.5, 5% Iso-Propanol, 10% PEG 4000

200 mM K Acetate, 25% peg 3350

200 mM K Acetate , 25% peg 3350

300 mM Na acetate, 25% peg 3350

200 mM Sodium formate, 25% PEG 3350

0. 300 M Lithium acetate, 25.0 % PEG 3350

0. 100 M Imidazole-HCl pH 8, 10% 2-propanol

0. 150 M Imidazole-HCl pH 8, 10% 2-propanol

Crystals formed within 1-7 days at 25 ºC, and were rod shaped in morphology.

The approximate cell dimensions of the crystals were a=77 Å, b=99 Å, c=129 Å, β=90 º. The space group is I222.

Following the above 30 (i.e. 1 + 29) conditions, the text at page 58 lines 24-25 continues:

Crystals of 3A4 were also grown over a reservoir solution containing:

0.15M HEPES pH7.5, 5% IPA, 10 % PEG 4000.

Further at page 59 lines 35-40:

Crystals of the 3A4 were grown using the hanging drop vapour diffusion method. Protein at 37.4 mg/ml in 20 mM Kpi pH 7.2, 0.5 M KCl, 2mM DTT, 1mM EDTA, 20% glycerol, was mixed in a 1:1 ratio, using 0.5ul drops, with a reservoir solution. The crystals of 3A4 grew over a reservoir solution containing 0.15 M HEPES pH 7.5, 2.5% IPA, 10% PEG 4000.

> Crystals formed within 1-7 days at 25 ºC, and were rod shaped in morphology.

Further still at page 61 lines 15-22:

> Crystals of the 3A4 were grown using the hanging drop vapour diffusion method. Protein at 18.5 mg/ml in 10 mM Kpi pH 7.2, 0.5 M KCl, 2 mM DTT, 1 mM EDTA, 20% glycerol, 10 mM K2SO4 was mixed in a 1:1 ratio, using 0.5ul drops, with a reservoir solution. The crystals of 3A4 grew over a reservoir solution containing 0.1 M HEPES pH 7.2, 5% IPA, 10% PEG 4000. The crystal was frozen using the crystallization solution supplemented by glycerol to 33%.

And finally at page 63 lines 10-14:

> Crystals of the 3A4 were grown using the hanging drop vapour diffusion method. Protein at 36 mg/ml in 10 mM Kpi pH 7.2, 0.5 M KCl, 2 mM DTT, 1 mM EDTA, 20% glycerol, was mixed in a 1:1 ratio, using 0.5 µl drops, with a reservoir solution. The crystals of 3A4 grew over a reservoir solution containing 0.1 M HEPES pH 7.5, 0.025 M sodium chloride, 7.5% IPA, 10% PEG 4000.

This makes a total of ***thirty four*** sets of conditions illustrated. The Examiner assertion that the specification teaches only two conditions is not believed to be consistent with a review of the application, as provided above.

It is also stated at page 13 lines 28-32 of the present application that:

> To the extent that the present invention relates to 3A4-ligand complexes and mutant, homologue, analogue, allelic form, species variant proteins of 3A4, crystals of such proteins may be formed. The skilled person would recognize that the conditions provided herein for crystallising 3A4 may be used to form such crystals.

This teaching is not mere speculation. In the field of P450 enzymes, it has been observed by those of skill in the art that once a set of conditions for crystallization of a

particular P450 protein has been determined, those conditions may be applied to its variants. This is illustrated by the P450 enzymes P450 BM3, P450cam, P450nor and P450 2C9, as further discussed in the following.

P450cam

The *Pseudomonas putida* cytochrome P450cam was crystallized by Poulos *et al* (1987).

Hishiki *et al* (2000)[1] describe preparation of crystals of a P450cam mutant, Thr252Ile, as well as crystals of wild-type enzyme.

Vidakovic *et al* (1998)[2] made a different mutant of P450cam, namely Asp251Asn.

Raag *et al* (1991)[3] made another mutant of P450cam, namely P450cam Thr252Ala.

The following table sets out the crystal cell and unit cell sizes of P450cam and its mutants cited above. For the convenience of the Examiner the Table includes the Protein Data Bank (PDB) file references:

Protein	PDB Ref	Crystal Form	*a*	*b*	*c*
WT	2CPP	P 21 21 21	108.670	103.900	36.380
Thr252Ile	1GEB	P 21 21 21	106.430	103.440	35.000
Thr252Ala	2CP4	P 21 21 21	108.0	104.2	36.2
Asp251Asn	6CP4	P 21 21 21	106.600	103.200	36.500

[1] Hishiki, T., *et al*: X-Ray Crystal Structure and Catalytic Properties of Thr252Ile Mutant of Cytochrome P450Cam: Roles of Thr252 and Water in the Active Center J.Biochem.(Tokyo) 128 pp. 965 (2000)

[2] Vidakovic, M., *et al*: Understanding the role of the essential Asp251 in cytochrome p450cam using site-directed mutagenesis, crystallography, and kinetic solvent isotope effect. Biochemistry 37 pp. 9211 (1998)

[3] Raag, *et al*: Crystal structure of the cytochrome P-450CAM active site mutant Thr252Ala. Biochemistry 30 pp. 11420 (1991)

Each of the unit cell dimensions are within a range of less than 5% from the lowest to the highest value.

The Examiner's attention is drawn to the fact that the WT crystal is described as being made by conditions taught by Poulos et al, (1982)[4] as being suitable for the production of an orthorhombic crystal of form P 21 21 21, a = 108.5, b = 104,4, c = 36.4. In Raag *et al* the authors state on page 11421 that

> *"P-450$_{CAM}$ was purified and crystallized according to procedures that have been described previously (Poulos et al., 1982;…."*

i.e. the same citation as given by Poulos *et al* (1987). On page 966 of Hisiki *et al* the authors state that:

> *"The wild-type and Ile252-mutant enzymes were crystallized as described by Poulos and his coworkers (26) with minor modifications".*

Reference 26 is Poulos *et al*, 1982, as cited above.

Thus the P450cam sequence variants not only have the same crystal form and substantially the same cell size, but those of skill in the art were able to make variants using methodology disclosed for the production of a wild-type form.

P450 BM3

Ravichandran *et al* (1993)[5] describe crystals of P450 BM3 wild-type heme domain. This protein comprises residues 1-472.

[4] Poulos. T.L., *et al:* Preliminary crystallographic data on cytochrome P-450CAM. J. Biol. Chem, 257; 10427-10429 (1982)

[5] Ravichandran, K. G., *et al*: Crystal structure of hemoprotein domain of P450BM-3, a prototype for microsomal P450's. Science 261 pp. 731 (1993)

In contrast, Li and Poulos (1995)[6] describe a crystal of the heme domain of bacterial P450, BM3. The protein consists of residues 1-455. This is a truncated version of the wild-type heme domain. The authors state that:

> "Molecular modelling using P450cam as a guide indicated that 1-455 could be generated without affecting the C-terminal β structure."

The protein comprises a C-terminal truncation of 16 amino acids compared to the 1-472 protein.

Ost *et al* (2001)[7] describes making a crystal of a P450BM3 mutant, F393H (which the Examiner will note incorporates a change of charge).

Ost *et al* (2003)[8] report three further mutants, F393A, F393W and F393Y.

The crystal forms and cell dimensions of the above P450 BM3 crystals are as set out in the following table, which also includes their PDB file references:

Protein	PDB Ref	Crystal Form	*a*	*b*	*c*	β
wt 1-472	2HPD	P21	59.40	154.00	62.20	94.70
wt 1-455	1BMH	P21	59.53	154.03	62.43	94.97
F393H	1JME	P21	58.84	153.10	61.48	94.67
F393A	1P0V	P21	58.69	152.91	61.20	94.64
F393W	1P0W	P21	58.91	153.54	61.43	94.42
F393Y	1P0X	P21	59.61	152.98	61.77	94.54

Each of the unit cell dimensions are within a range of less than 5% from the lowest to the highest value.

---

[6] Li H, Poulos TL: Modeling protein-substrate interactions in the heme domain of cytochrome P450(BM-3). Acta Crystallogr D Biol Crystallogr. 1995 Jan 1;51(Pt 1):21-32

[7] Ost, T. W. B., *et al*: Structural and Spectroscopic Analysis of the F393H Mutant of Flavocytochrome P450 Bm3 Biochemistry 40 pp. 13430 (2001)

[8] Ost, T. W. B., *et al*: Oxygen Activation and Electron Transfer in Flavocytochrome P450 Bm3 J.Am.Chem.Soc. 125 pp. 15010 (2003)

The Examiner's attention is further drawn to the fact that Ramachandran produced his crystals as described in Boddupalli *et al* (1992)[9] using 100mM Pipes, 20% (wt/vol) PEG 8000, 15 mM DTT, and 40mM $MgSO_4$ at pH 6.8. Li and Poulos stated that 'crystallisation condition were similar to those of Boddupalli *et al* (1992) with some modification' (18% PEG 8000, 50 mM $MgSO_4$ and 100 mM PIPES pH6.8 or 16% PEG 8000, 100 mM MgSO4 and 100 mM PIPES pH6.8). In addition, substantially the same conditions were also used to crystallize the four Ost mutants (18-21% PEG 8000, 40 mM $MgSO_4$ and 100 mM PIPES pH6.5-7.5).

Thus variants of the WT1-472 protein which have a change in sequence - including a charge change crystallize under substantially the same conditions and produce a product of the same crystal form and unit cell size.

Further variants of the WT1-472 protein which have a C-terminal truncation crystallize under substantially the same conditions and produce a product of the same crystal form and unit cell size.

P450nor

The P450 protein, P450nor, has been crystallized by Shimizu *et al*, (2000)[10]. Also described in the reference are the variants S286V and S286T. Obayashi *et a*,

[9] Boddupalli S.S., *et al:* Crystallization and preliminary x-ray diffraction analysis of P450terp and the hemoprotein domain of P450BM-3, enzymes belonging to two distinct classes of the cytochrome P450 superfamily. Proc Natl Acad Sci U S A. 89:5567-71 (1992)

[10] Shimizu, H., *et al*: Crystal Structures of Cytochrome P450Nor and its Mutants (Ser286 Val, Thr) in the Ferric Resting State at Cryogenic Temperature: A Comparative Analysis with Monooxygenase Cytochrome P450S, J.Inorg.Biochem. 81 pp. 191 (2000)

(2000)[11] describe three further variants of P450nor, i.e. T243N (which introduces a change of charge), T243A and T243V.

The crystal forms and cell dimensions of the above P450nor crystals are as set out in the following table, which also includes their PDB file references.

Protein	PDB Ref	Crystal Form	*a*	*b*	*c*
WT	1EHE	P 21 21 21	54.507	81.768	85.759
S286V	1EHG	P 21 21 21	54.751	81.860	85.720
S286T	1EHF	P 21 21 21	54.559	81.728	85.737
T243N	1F25	P 21 21 21	54.56	81.92	85.70
T243A	1F24	P 21 21 21	54.58	81.76	85.64
T243V	1F26	P 21 21 21	54.57	81.94	85.53

Each of the unit cell dimensions are within a range of less than 5% from the lowest to the highest value.

The Examiner's attention is further drawn to the fact that Shimizu *et al* state that all their crystals were made under the same conditions as for the crystallization of the native fungal enzyme (page 192, r.h. column), whereas Obayashi *et al* merely cite Shimizu *et al* (reference [14] cited at page 104, section 2.1) for crystal preparation. Thus the P450nor sequence variants not only have the same crystal form and substantially the same cell size, but those of skill in the art were able to make variants using methodology disclosed for the production of a wild-type form.

P450 2C9

The attached Declaration from Dr. Williams refers to the crystallization of various clones of the human P450 2C9.

[11] Obayashi, E., *et al*: Mutation Effects of Conserved Threonine(Thr243) of Cytochrome P450Nor on its Structure and Function, J.Inorg.Biochem. 82 pp. 103-111 (2000)

From the attached Declaration of Dr. Williams (paragraph 10), the unit cell sizes of the P450 2C9 mutants were as follows:

2C9 Clone	Unit Cell	a	b	c
1015	P321	161.35	161.35	110.75
1015	P321	163.95	163.95	111.06
1155	P321	165.46	165.46	111.70
1155	P321	164.87	164.87	111.11
1475	P321	165.39	165.39	110.91
1491	P321	164.80	164.80	111.23
1982	P321	165.31	165.31	111.45
1983	P321	164.57	164.57	111.25

2C9 clone 1015 comprises 6 mutations over wild-type 2C9, i.e. I215V C216Y S220P P221A I222L and I223L. The clone also comprises a truncated N-terminal region to delete the hydrophobic trans-membrane domain which is replaced by a short amino acid sequence (MAKKTSSKGR in place of the N-terminal 29 amino acid residues) and a C-terminal histidine tag.

2C9 clone 1155 is as clone 1015 with the additional mutation of K206E.

2C9 clone 1475 is as clone 1155 with the additional mutation of Asn231His.

2C9 clone 1491 is as clone 1155 with the additional mutation of L208A.

2C9 clone 1982 is as clone 1155 with the additional mutation of A103Y.

2C9 clone 1983 is as clone 1155 with the additional mutation of A103W.

As can be seen from the above table, a range of P450 2C9 variants have the same unit cell form and have sizes within 5% of each other. Some of the above clones are described in WO03/035693 (copy previously submitted). This citation describes numerous crystallization conditions for 2C9 proteins of varying sequences. One of the conditions described is 0.1-0.4 (particularly 0.2) M KH_2PO_4, 0-25% (particularly 20%)

PEG 3350, 0-10% (particularly 0%) glycerol. The crystallization of clone 1115 under these conditions is taught at page 61 lines 35-36 and the crystallization of clone 1015 under these conditions is taught at page 57 line 2.

The claims are supported by an adequate written description.

In reconsidering the written description support of the present application, the Examiner is requested to see the CAFC decision in *Capon v Eshhar,* Case 03-1480, -1481 (Interference No. 103,887), copy attached. In Capon v Eshhar, the applicants were claiming chimeric genes based upon fusions of known classes of genes. The Patent Office Board of Appeals and Interferences (i.e., the Board) held that the claims failed to be supported by an adequate written description in that the applications of each party allegedly failured to report and analyze each and every gene of the classes.

In overturning the Board, the CAFC held:

> The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science. …
>
> The "written description" requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution.

In applying the reasoning of the CAFC Decision to the facts of the present application, the present applicants submit that the individual components of crystallization buffers are known chemical compounds which are widely used by those of ordinary skill in the art to produce protein crystals. The present inventors have discovered an appropriate set of conditions for the crystallization of 3A4 proteins and described in the present application particular combinations of ranges of such compounds which are suitable for the production of 3A4 protein crystals.

As illustrated above, it is known in the art for other P450 members that variants of such members are able to crystallize under substantially identical conditions to a wild-type sequence, and form crystals of the same unit cell size and form. It is also known in the art that short stretches of amino acid sequences outside the core region of a P450 protein do not affect the size or form of crystals of that protein, i.e. the BM3 wt 1-472 protein has an additional 16 amino acids at its C-terminus compared to the core BM3 wt 1-455. Both are reported to crystallize under substantially the same conditions and to give the same unit cell size. This is in keeping with what a ordinarily skilled person would understand as generally applicable to P450 protein crystals, as set out in the Declaration of Dr. Williams at paragraphs 12-17.

In summary, the ordinarily skilled artisan would understand from the teaching of the present specification, in the light of the knowledge of the P450 crystals set out above, that the conditions used for the crystallization of a particular member of the P450 family, once determined, may be applied to variants of that family including variants comprising point mutations and sequence truncations, as set out in the written description of the present application.

The claims are supported by an adequate written description.

Enablement

The claims are supported by an enabling disclosure. One of ordinary skill in the art will be able to make and use the claimed invention without undue experimentation.

The Examiner has stated that the claims :

> "... are broader than the enablement provided by the disclosure with regard to all crystals comprising a any [sic] P450 3A4 from any mammal or modified form thereof ..." (see page 3 of the Office Action dated July 18, 2005).

As noted above, the protein P450 3A4 is a human protein, and is not present in other mammals. Thus the protein does not extend to such non-human mammalian proteins by virtue of the terminology recognized by those of ordinary skill in the art. See attached Declaration of Dr. Williams, paragraph 7..

The Examiner has further stated that the claims extend to any modified form of P450 3A4 and may include:

> "... that may include any insertion, deletion, substitution or combination thereof mutants and fragments thereof expressed in any host cell." Id.

None of the pending claims 102-117 are directed to mutants by insertion, deletion or substitution of the recited core 3A4 sequence of SEQ ID NO:2.

Claim 102 is directed to a crystal of P450 3A4 which comprises a core of residues 17 to 476 of SEQ ID NO:2, with a specified crystal form and unit cell size. Residue 476 is the last resolvable residue at the C-terminus of the protein. As noted in

the attached Declaration of Dr. Williams, N- and C-terminal tails outside a core region are unlikely to influence the structure of the more rigidly folded domains of the 3A4 protein itself. See, Williams Declaration, paragraph 16. Further, as evidenced by Ravichandran *et al* (1933), the presence of residues outside the core region of a P450 does not alter the crystal size or form of the core protein.

Claims 103-109 set out preferred features of the N- and C-terminal regions.

Claim 110 is directed to a crystal of the core protein with a C-terminal extension no larger than 10 amino acids and wherein the N-terminal region which comprises a truncation by deletion of the 3A4 transmembrane domain. The protein of SEQ ID NO:2 comprises such a truncation. As noted in the attached Declaration of Dr. Williams (paragraph 16), minor variations of this nature in the N- or C-terminal regions are unlikely to affect the structure of the more rigidly folded domains.

Claims 111-113 are directed to specifically preferred features of this aspect of the invention.

Claim 114 is directed to a crystal of P450 3A4 comprising a core of residues 3 to 476 and wherein the core comprises an N- region of smaller than 20 amino acids and a C-terminal region no larger than 10 amino acids.

Claims 115 and 116 are directed to specifically preferred features of this aspect of the invention.

Claim 117 is directed to a crystal of SEQ ID NO:2, indicated to be allowable subject matter by the Examiner.

In summary, none of the pending claims 102-117 are directed to mutants by insertion, deletion or substitution of the recited core 3A4 sequence of SEQ ID NO:2. The claims are supported by an enabling disclosure.

Claim 118 is directed to a crystal of SEQ ID NO:2 or a protein which comprises 1 or 2 amino acid insertions or deletions thereof, which are typical of many of the alleles of 3A4 known in the art (see description at page 13 lines 24-26). As indicated above, it is known in the field of P450 proteins that a small number of changes to the protein primary sequence can be made without affecting the overall crystal unit cell size and crystal form of that protein. In the case of 2C9, clone 1015 comprises 6 alterations from the wild-type sequence (in addition to an N-terminal truncation and a C-terminal His tag), clone 1155 comprises one additional alteration, and clones 1475, 1491, 1982 and 1983 two additional alterations. These alterations to 1015 resulted in a protein which provided a crystal of the same unit cell size and same crystal form.

In view of the teaching of the art in relation to P450 crystal structures, it is submitted that the experimentation required to provide crystals according to the present claims is far from undue.

With regard to the "Wands factors" referred to by the Examiner on page 3 of the Office Action, the Examiner is requested to consider the following.

(a) The experimentation necessary to produce variants (point mutations or sequence truncations) of a protein is routine in the art – as set out in the present application at page 13 lines 8-13, commercially available kits for site-directed mutagenesis are available, and these techniques are taught in generally available textbooks known to those of skill in the art.

(b) The amount of guidance presented for the formation of crystals is extensive. As noted above, there are 34 specific sets of conditions suitable for crystallization of 3A4, in addition to extensive generic teaching. Those of skill in the art familiar with crystals of other P450 proteins would reasonably expect such conditions to be applicable to the variants of SEQ ID NO:2 recited in claim 118.

(c) The specification presents a working example of a 3A4 P450 crystal of SEQ ID NO:2. For the reasons explained above and in the attached Declaration of Dr. Williams (see in particular paragraphs 11, 12, 15 and 16), those of ordinary skill in the art would have a reasonable expectation that the example is illustrative of the other embodiments of 3A4 sequences claimed.

(d) The nature of the invention is a crystal of a P450 family member. Crystallization of other P450 family members are known and those of skill in the art would read, understand and interpret the specification in this light.

(e) The state of the prior art, as set out above, provides sound basis for the person of ordinary skill in the art to not require undue experimentation for the provision of crystals of sequence variants of SEQ ID NO:2 as claimed (Williams Declaration paragraphs 11-12).

(f) The relative skill of those who practice in the art is illustrated by the attached Declaration of Dr. Williams, plus the art relating to P450 BM3, P450nor and P450cam crystals. The practitioners crystallizing P450 variants of a particular family member were able to make variants of their respective P450 family members without undue difficulty.

(g) The evidence in relation to P450 family members shows that once a particular P450 family member has been crystallized, it is reasonably predictable that minor variants of that family member will be obtained without undue difficulty or experimentation. The fact that this has occurred on four previous occasions provides sound basis for predictability for the present invention once one is provided with the teaching of the present application.

(h) The claims are of a reasonable breadth. Claims 102-117 relate to the invariant core sequence of SEQ ID NO:2, and claims 118 and 119 relate to crystals of the whole of SEQ ID NO:2 with only minor modifications. Up to 2 amino acid variations in a sequence of 485 amino acids is a variation of only less than 0.5%, which is within the scope of known P450 alleles (see Williams Declaration, paragraph 17). It is commensurate with the experience of the prior art in the field of P450s, and that of Dr. Williams in relation to P450 2C9, that crystals within this scope of variation form under substantially the same conditions and produce the same crystal form and unit cell size for any given P450 protein. The claims are a reasonable extrapolation which, based on the experience of those working in the art, an ordinarily skilled artisan would envisage.

For completeness, the applicants note that the Examiner has referred to the section of the present application at pages 3-4 entitled "Background to Crystallization" to allegedly support the Examiner's enablement rejection. The applicants note however that this background section is written with reference to crystallization of proteins of unknown structure for the first time i.e. novel structures, not of the same protein in which minor modifications to small N- and C-terminal regions once the structure has been solved or to the primary sequence (which do not appear to form significant stable

secondary or tertiary structures in any event) may occur. The Examiner is directed to the attached Williams Declaration, paragraph 20, where Dr. Williams states that the Examiner's position is a mis-representation of the state of the art.

The Examiner has further asserted that:

> "The amount of experimentation to identify crystallization conditions from any particular P450 3A4 from any biological source as well as modified form thereof and expressed in any host cell and identifies [sic] a crystal suitable for structure determination by X-ray crystallography is enormous"

The Examiner provides no citation of technical literature in support of this assertion. The Examiner is requested to provide some support for the assertion in the event the contention is maintained in rejecting the claims for an alleged lack of enabling and/or written description support.

As previously explained, P450 3A4 is a human protein, and in view of this reference to "any biological source" is believed to be misplaced. Equally, reference to expression in any host cell is irrelevant to the claims in question, particularly as 3A4 is a non-glycosylated protein. As to whether or not the experimentation required is "enormous", the evidence from other P450 proteins is to the contrary. For completeness, the applicants further note that the amount of experimentation is insignificant in determining whether undue experimentation is required to make and use the claimed invention. The issue for enablement is whether any required experimentation is undue.

The Examiner has further set out in his rejection that:

> "Since routine experimentation in the art does not include screening large number [sic] of

> crystallization conditions for the wild-type P450 3A4 or modified form thereof which can be crystallized where the expectation of obtaining the desired crystal is unpredictable, the Examiner finds that one skilled in the art would require additional guidance, such as information regarding the exact crystallization conditions that produce a crystal suitable for structure determination by X-ray crystallography and having the crystal parameters sited [sic] in claims 98 and 101."

It is of course an element of the present invention that the inventors have devised conditions suitable for the crystallization of a 3A4 protein of SEQ ID NO:2. While the art does not provide such conditions, it is a premise of the present invention, based upon the evidence referred to above, that having devised numerous specific conditions suitable for crystals of SEQ ID NO:2, those of ordinary skill in the art will reasonably expect to apply such conditions to the production of variants of SEQ ID NO:2 within the scope of the present claims. As noted above with P450cam, neither Raag *et al* (1991) or Hishiki *et al* (2000) required anything other than the established teachings of Poulos (1982) to produce crystals of P450cam variants. Similarly, Ost *et al* (both 2001 & 2003) and Li & Poulos (1996) were able to follow essentially the conditions of Boddupalli *et al* (1992) for crystallization of mutants of P450 BM3. Variants of P450nor were all made as that of the native enzyme.

It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. See In re Angstadt, 537 F.2d 498, 504 (CCPA 1976) ("The examples, both operative and inoperative, are the best guidance this art permits, as far as we can conclude from the record"). It is

submitted that the generic teaching and the provision of ***thirty four*** specific sets of conditions of the present application illustrates that the inventors have provided adequate written description and enabling support of the present invention in the light of the state of the art for P450 proteins.

In the present invention, an illustrated embodiment is a 3A4 P450 comprising a truncated N-terminal region and a poly-histidine tag at its C-terminus. It is also taught that variants of 3A4 proteins are known in the art, including about 25 naturally occurring alleles (description at page 13 line 24). The present inventors do not claim to have invented alleles of 3A4, nor any particular C-terminal tag. The truncation of an N-terminal region, was known (Gillam *et al*, Arch. Biochem. Biophys. Vol. 305, 123-131, 1993, previously submitted) . What the inventors have contributed to the art is the teaching of crystals comprising the 3A4 core region. Based on the current scientific and technical knowledge of P450 proteins in the art, the teaching of the present application fairly describes how to make protein crystals of the same unit cell structure which comprise this core or its variants (as defined in the claims).

In other words, the "scientific and technologic knowledge" already in the art includes the knowledge of crystallization of variants of proteins and crystallization of proteins comprising a P450 core together with additional non-core sequences. By the reasoning expressed by the CAFC in the Decision of Capon v Eshhar, the present Examiner should not impose a written description standard on the present applicants which fails to take account of the current knowledge of the state of the art, i.e. including known variants of 3A4 and known methods of N- and C-terminal modifications. Rather, the present invention resides in the crystallization of 3A4 proteins having a core region

which produce crystals of a specified unit cell size. The core region (about 95% of the 3A4 protein of SEQ ID NO:2) will, on the basis of the results with three other P450 isoforms, be reasonably expected to retain an overall fold and structure for the particular 3A4 illustrated in the present specification and can be obtained following the extensive written description set out in the present application.

Once a person of ordinary skill in the art has invented a set of conditions to achieve crystallization of a particular P450 isoform, it is established in the art that such conditions are suitable for the production of variants of that isoform.

It is thus submitted that having devised the conditions for the crystallization of P450 3A4, no undue experimentation is required for the production of the variants set out in the present claims. No evidence has been cited by the Examiner to support his assertions to the contrary, and in the light of the evidence above and attached, the claims are submitted to be supported by an enabling disclosure.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned in the event anything further is required in this regard.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
B. J. Sadoff
Reg. No. 36,663

BJS:
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100